I / We Claims

1. An oligonucleotide primer pair having SEQ ID NO: 3 and SEQ ID NO: 4 for amplification of Early Secretory Antigenic Target (*esat*)-6-gene of *Mycobacterium* species.

2. A method for detecting *M .tuberculosis* in a sample based on the amplification of *esat*-6 gene, the said method comprising the steps of :
   a) isolating DNA template from the sample,
   d) amplifying the DNA template by adding a reaction buffer, oligonucleotide primer pair having SEQ ID NO: 3 and SEQ ID NO: 4, and heat stable DNA polymerase to obtain an amplified DNA product, and
   e) subjecting the amplified DNA product of step (b) to separation, and staining to detect the presence of amplified DNA product wherein the presence of amplified DNA product is indicative of *Mycobacterium tuberculosis* in the sample.

3. A method according to claim 2, wherein the sample is either clinical sample or culture sample.

4. A method according to claim 3, wherein the clinical samples is selected from a group of sputum, bronchoalveolar, lavage fluid, pleural fluid, ascetic/peritoneal fluid, cerebrospinal fluid (CSF), pus fecal matter, urine, amniotic fluid, menstrual blood, peripheral blood or other body fluids, lymph node, pus or other aspirate, and tissue biopsies.

5. A method as claimed in 2 wherein in step (b) the amplification is by polymerase chain reaction.

6. A method as claimed in 2 wherein the amplification consists of 25-35 cycles of amplification.

7. A method according to claim 2, wherein in step (b) the heat stable DNA polymerase is *Taq polymerase*.

8. A method as claimed in 2 wherein in step (c) the separation is done preferably by gel electrophoresis.

9. A method as claimed in 2 wherein in step (c) the staining is by ethidium bromide.

10. A method as claimed in 2 wherein in step (c) the amplified DNA product is 320 base pair in length.

11. A diagnostic kit for detection of *Mycobacterium tuberculosis*, from other species of *Mycobacteria* comprising of oligonucleotides primers having SEQ ID NO: 3 and SEQ ID NO: 4, all four deoxyridonucleotide triphosphate (dNTPs), reaction buffer, *Taq polymerase*, DNA marker, positive and negative control and instruction manual.

12. A method for detecting *M. tuberculosis* based on amplification wherein, the said method comprising the steps of:

i. amplifying the 16s rRNA region from the isolated DNA template using the primer pair having SEQ ID NO: 1 and SEQ ID NO: 2 to obtain first amplified product using conventional method,

ii. detecting the amplified product of step (a) wherein the presence of 1030 base pair amplified DNA product is indicative of positive sample for the presence of *Mycobacterium* species,

iii. employing the DNA from the positive samples identified from step (b) for further detection of *M. tuberculosis* based on the amplification of *esat*-6 gene,

iv. amplifying the *esat*-6 gene using the primer pair having SEQ ID NO: 3 and SEQ ID NO: 4 to obtain second amplified product using method as claimed in claim 2, and

v. detecting the amplified product of step (d) wherein the presence of 320 base pair is indicative of *Mycobacterium tuberculosis* in the sample and absence is indicative of other *Mycobacterium* species.

13. A method according to claim 12, wherein the DNA template is obtained either from clinical sample or from culture sample.

14. A method according to claim 13, wherein the clinical sample is selected from a group of sputum, bronchoalveolar, lavage fluid, pleural fluid, ascetic/peritoneal fluid, cerebrospinal fluid (CSF), pus fecal matter, urine, amniotic fluid, menstrual blood, peripheral blood or other body fluids, lymph node, pus or other aspirate, and tissue biopsies.

15. A method as claimed in 12 wherein the amplification is by polymerase chain reaction.

16. A method according to claim 15 wherein the amplification is by heat stable DNA polymerase such as *Taq polymerase*.

17. A method as claimed in 12 wherein in step (i) the amplification consists of 30-40 cycles of amplification.

18. A method as claimed in 12 wherein in step (iii) the amplification consists of 25-35 cycles of amplification